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Histone Deacetylase Inhibitors: Synthesis of Tetrapeptide Analogue SAHA/TPX

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Abstract: The inhibition of HDAC (histone deacetylase) activity by specific inhibitors induces growth arrest, differentiation and apoptosis of transformed or several cancer cells. Some of these inhibitors are in clinical trial at phase I or phase II. The discovery and development of specific HDAC inhibitors are helpful for cancer therapy. In this paper we describe the synthesis of simple inhibitor **B** hybrid analogue suberoylanilide hydroxamic acid (SAHA), trapoxin **B** (TPX B) in as little as five steps. This compound is interesting lead for the design of potent inhibitors of histone deacetylase.

Keywords: Histone deacetylase inhibitors, Tetrapeptide, Hydroxamic acid

Introduction

Inhibitors of histone deacetylase (HDACs) are a relatively new class of potential drugs for the treatment of hyperproliferative diseases[1-3]. They bind directly to the HDAC active site and block substrate access, causing an accumulation of acetylated-histone[4-7]. These agents possess diverse biological activities and can affect differentiation, growth arrest and / or apoptosis in transformed cell cultures[8,9]. HDAC have emerged as an attractive target for new anticancer drugs[10,11] and there is a great demand for new inhibitors. Several families of small potent (IC_{50} < 100 nM) molecule HDAC inhibitors have been reported in the recent literature[12-15] (Figure 1). Suberoylanilide hydroxamic acid (SAHA) (**2**), one of the reversible inhibitors bearing a hydroxamic acid and an aromatic terminus group separated by a hydrophobic spacer (lit. IC_{50}.10 nM)[16,17], cyclic tetrapeptide trapoxin **B** (TPX B) (**3**), an

irreversible inhibitor from the cyclic tetrapeptide family which has an epoxyketone at the terminus of the hydrophobic chain (lit. IC_{50} 0.10 nM)[18] and CHAP 31 (**6**) hybrid analogue of the cyclic peptide and hydroxamic acid (lit. IC_{50} 2 nM)[19].

The basic structure of these inhibitors mimics trichostatine (TSA) (**1**) in that they possess a cap group, an aliphatic chain for a spacer, and a functional group[20]. These inhibitors contain several functional groups that potentially interact with the active-site, including hydroxamic acids, carboxylic acids, phenylene diamines, and epoxyketones[21]. The cap group may be necessary for packing the inhibitor at the rim of the tube-like active-site pocket[22]. Whereas the aliphatic chain mimics that of lysine and forms van der waals interactions with the residues lining the pocket[23,24]. Recently some novel synthetic inhibitors have been developed based on this structural information. Here we propose a general structure for analogue **B**. In order to find a novel hydroxamate Inhibitors of histone deacetylase, we set out to combine various elements from **2** and **3** (Figure 1).

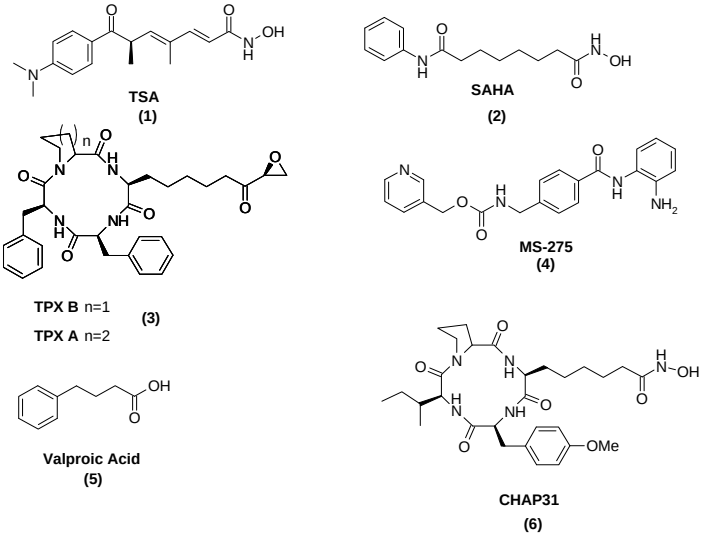


Figure 1. Structures of HDAC inhibitors

Experimental

Nuclear magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) were recorded on a Bruker 300 and 75 MHZ. Flash column chromatography was performed using MACHEREY-NAGEL silica gel 60 (15-40 µm) as the stationary phase. All reactions were run under a positive pressure of nitrogen unless otherwise started.

*[1-Benzylcarbamoyl-2-(4-hydroxy-phenyl)-ethyl]-carbamic acid tert-butyl ester (**9**)*

To a solution of boc-*L*-tyrosine acid **7** (1 eq, 10.6 mmol) in dimethylformamide (DMF) (28.4 mL) were added 1-[3(dimethylamino)propyl]-3-ethyl-carboimide hydrochloride (EDC) (1.5 eq, 15.9 mmol), *N*-hydroxybenzotriazole (HOBT) (1.3 eq, 13.78 mmol). The mixture was stirred 1 h at room temperature. Benzylamine (**8**) (1 eq, 10.6 mmol), triethylamine (TEA) (1 eq, 1.47 mL) were added and the mixture was stirred for 2 days. A saturated $NHCO_3$ solution was added and the phases were separated. The organic layer was extracted with a mixture (Ethyl acetate/Benzene, 2/1) and the combined organic fractions were dried over $MgSO_4$ then evaporated. The crude product was purified by flash chromatography (MeOH/CH_2Cl_2, 3/97) to give compound **9** (90%, viscous oil). ^{1}H NMR

($CDCl_3$) δ ppm 1.39 (s, 9H), 2.97 (m, 2H), 4.32 (m, 3H), 5.16 (sl, 1H), 6.27 (sl, 1H), 6.67 (d, *J* = 8.43 Hz, 2H), 6.96 (d, *J* = 8.25 Hz, 1H), 7.20 (m, 5H). ^{13}C ($CDCl_3$) NMR δ ppm 37.7, 43.4, 56.6, 77.0, 116.0, 127.0, 130.0, 128.6, 129.0, 137.4, 155.1, 155.5, 171,4.

*2-Amino-N-benzyl-3-(4-hydroxyphenyl)-propionamide (**10**)*

The *N*-protected group (**9**) (3.55 g, 9 mmol) was dissolved in trifluoroacetic acid (TFA) (16.4 mL). The mixture was stirred for 3 h at room temperature. The residue was concentrated under reduced pressure and then neutralized with saturated Na_2CO_3. The resulting solution was extracted 3 times with ethyl acetate (20 mL). The combined organic layers were washed with water then brine, dried over Na_2SO_4 and concentrated in vacuum. The crude product was purified by flash chromatography (Ethyl acetate /Petroleum ether, 4/98) to give compound **10** (97%, white solid). ^1H NMR (CD_3 OD) δ ppm 2.78 (m, 2H), 3.53 (t, *J* = 6.9 Hz, 1H), 4.23 (d, *J* = 14.94 Hz, 1H), 4.35 (d, *J* = 14.94 Hz, 1H), 6.66 (d, *J* = 8.5 Hz, 2H), 6.69 (d, *J* = 8.47 Hz, 2H), 7.07 (d, *J* = 8.10 Hz, 1H), 7.21 (m, H). ^{13}C (CD_3OD) NMR δ ppm 42.1, 44.3, 58.4, 116.7, 128.5, 128.9, 129.6, 129.8, 131.8, 139.8, 157.9, 176.9.

*7-Phenoxycarbamoyl-heptanoic acid (**13**)*

The suberic acid **11** (1 eq, 5.7 mmol), 1-[3(dimethylamino)propyl]-3-ethyl-carboimide hydrochloride (1.5 eq, 8.55 mmol), *N*-hydroxybenzotriazole (1.3 eq, 7.41 mmol) were reacted in dimethylformamide (15.6 mL) for 1 h. *O*-benzyl hydroxylamine hydrochloride (**12**) (1 eq, 5.7 mmol), triethylamine (1 eq, 0.79 mL) were added and the mixture was stirred for 2 days. The resulting solution was acidified with 1N HCl and then extracted with mixture of (Ethyl acetate /Benzene, 2/1). The combined organic layers were dried and then concentrated under reduced pressure. Purification by flash chromatography ($MeOH/CH_2Cl_2$, 2/98) offered compound **13** (95%, colorless oil). ^1H NMR (CD_3OD) δ ppm 1.23 (m, 4H), 1.53 (m, 4H), 2.02 (m, 2H), 2.33 (m, 2H), 4.80 (sl, 2H), 7.26 (m, 5H), 9.18 (sl, 1H). ^{13}C (CD_3OD) NMR δ ppm 24.9, 25.6, 25.6, 29.0, 33.0, 34.4, 78.4, 112.6, 115.8, 126.7, 128.9, 129.4, 138.3, 163.7, 178.6.

*Octanedioic acid [1-benzylcarbamoyl-2-(4-hydroxy-phenyl)-ethyl]-amide phenoxy-amide (**14**)*

To a solution of amine (**10**) (1 eq, 0.74 mmol) and carboxylic acid (**13**) (1.05 eq, 0.77 mmol) in dimethylformamide (1.75 mL) were added portion wise of diphenylphosphorylazide (DPPA) (1.1 eq, 0.176 mL), triethylamine (2.15 eq, 0.22 mL) at 0 °C. The reaction continued overnight at room temperature. The solvent was removed under reduced pressure. The residue was purified by flash chromatography ($MeOH/CH_2Cl_2$, 4/96) to give compound **14** (67%, yellow solid). ^1H NMR (DMSO-d_6) δ ppm 1.12 (m, 4H), 1.37 (m, 4H), 1.90 (m, 2H), 2.01 (m, 2H), 4.23 (sl, 2H), 4.27 (m, 1H), 4.77 (sl, 2H), 6.16 (d, *J* = 9.78 Hz, 2H), 7.12 (d, *J* = 6.93 Hz, 2H), 7.25 (m, 10H), 8.02 (d, *J* = 9.78 Hz, 1H), 8.44 (sl, 1H), 9.20 (sl, 1H). ^{13}C (DMSO-d_6) NMR δ ppm 25.1, 25.4, 28.5, 32.5, 35.5, 37.4, 42.2, 54.6, 77.0, 115.1, 121.6, 126.9, 128.3, 130.4, 136.4, 139.5, 156.0, 169.6, 171.7, 172.3.

*8-Oxo-nonanoic acid [1-benzylcarbamoyl-2-(4-hydroxy-phenyl)-ethyl]-amide **B***

The *N*-Benzyl precursor (128 mg) **(14)** was dissolved in methanol (3.29 mL), catalytic amount of 5% Pd-$BaSO_4$ was then added and the solution was shaken under H_2. After 4 h the mixture was filtered through celite and the filtrate was evaporated. The hydroxamic acid **B** was isolated after purification of small sample on preparative plates (52%, white solid) ^1H NMR (CD_3OD) δ (ppm) 1.18 (m, 4H), 1.37 (m, 4H), 1.93 (m, 4H), 2.04 (d, 1H, *J* = 7.35 Hz),

2.92 (d, 1H, *J* = 7,35 Hz), 4.18 (q, 2H, *J* = 16.98 Hz), 4.47 (d, 1H, *J* = 1.32 Hz), 6.57 (d, 2H, *J* = 8,49 Hz), 6.92 (d, 2H, *J* = 8.46 Hz), 7.02 (d, 2H, *J* = 1.5 Hz), 7.11 (m, 3H). ^{13}C (CD_3OD) NMR δ ppm 26.9, 27.1, 30.0, 34.0, 37.1, 38.7, 44.4, 56.8, 116.7, 128.5, 131.7, 129.4, 139.8, 157.6, 173.4, 174.1, 176.5.

Results and Discussion

In the course of our studies to design and develop new inhibitors of HDAC, we wanted to combine structural elements of suberoylanilide hydroxamic acid (SAHA) and trapoxin **B** (TPX B) to get a simple and strong inhibitor of histone deacetylase (Figure 2), as so far only a few tetrapeptide analogues.

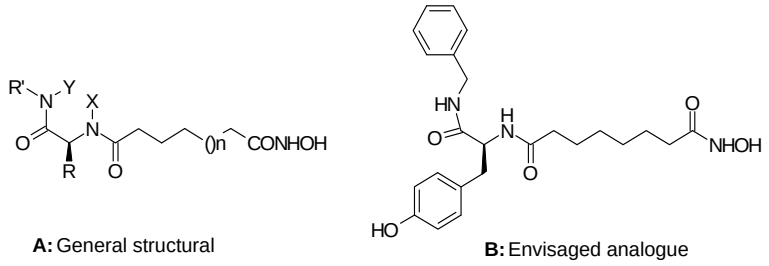
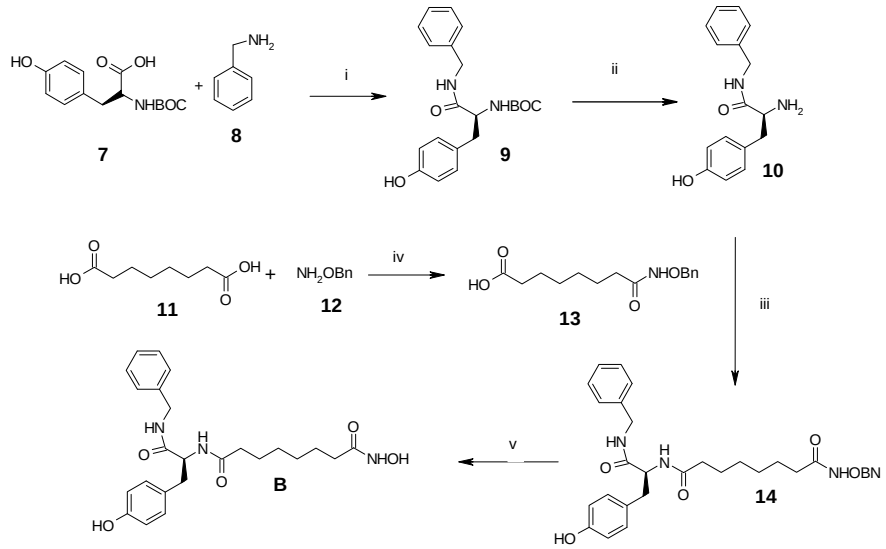


Figure 2. Envisaged hybrid analogue SAHA/TPX B

A modification of the spacer was not undertaken at this point, as it is known from the SAHA, that suberoyl compounds display the peak of activity[25]. In this study we focus the research on the region responsible for selective binding to the enzyme and we have modified the peptide portion (cap group) of the molecule **B** (Figure 2). For this synthesis we chose suberic acid as basic structure of length chain. The synthesis of this compound is illustrated in Scheme 1.



Scheme 1. General synthetic route analogue **B**

Reagents: (i) EDC, HOBT, TEA, 90%, (ii) TFA, 97%, (iii) DMF, DPPA, TEA, 67%, (iv) EDC, HOBT, TEA, 95%, (v) H_2/Pd-$BaSO_4$, MeOH

Our synthesis began with the coupling of the boc -*L*-tyrosine acid **7** and benzylamine **8** in the presence of triethylamine, under a standard mediate coupling condition (1-[3(dimethyl-amino)propyl]-3-ethyl-carboimide hydrochloride, *N*-hydroxybenzotriazole) to give the corresponding amide **9** in nearly quantitative yield. The *N* protecting group was removed under acidic condition to expose the primary amine **10**. The condensation of the amine **10** with acid carboxylic **13** in the presence of diphenylphosphorylazid gave the precursor hydroxamic acid **14** in 67% yield. Subsequent catalytic hydrogenation of the compound **14** using Pd/C was not viable, so we obtained complex and polar mixture that we were unable to isolate. Finally, the use of $Pd/BaSO_4$ (5%) as catalytic reagent gives the desired analogue **B** in 52% yield. The suberic acid **11** was converted to the protected hydroxamate **13** under typical peptide coupling conditions (1-[3(dimethylamino)propyl]-3-ethyl-carboimide hydrochloride, *N*-hydroxybenzotriazole). This synthetic route allowed for the synthesis of a large number of hydroxamic acid analogues.

Conclusion

In this paper we have synthesized a new class of HDAC inhibitors analogue hybrid SAHA/TPX B, the work has focused on the region responsible for selective binding to the enzyme (cap group). This analogue can allow for the synthesis of a large number of hydroxamic acid inhibitors.

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